



Amendments to application: DE NOVO SYNTHESIZED PLASMID, METHODS OF MAKING AND USE THEREOF

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a.) Introductory Comments

These are amendments to application (Application Number: 10/068,664) filed on February 6, 2002.

The claim(s) contains subject matter which was described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

Twelve copies of original Oligo Data Sheet of synthesized oligos corresponding to sequence ID NOS 1 to 12 are provided with this amendment. The sequence ID NOS are labeled on the top of each of the Oligo Data Sheet. Five copies of 5 relevant pages of the original notebook are also provided with this amendment. The relevant pages of the original notebook were witnessed, understood, and signed by different scientists. The first 2 pages of the copies of the original notebook demonstrate the synthesis of the DNA fragments containing replication origins and selection marker using different plasmid DNA such as pBR322, pUC19, pACYC177, and pACYC184 as templates by PCR. The DNA fragment of replication origin 4 of claimed plasmid p4C is shown on lane 5 of the first picture of page 2 of the copies of the original notebook. The DNA fragment of chloramphenicol selection marker gene of the claimed plasmid p4C is shown on lane 15 of the first picture on page 2 of the copies of the original notebook. The last 3 pages of the copies of the original notebook show evidence that the applicant made and had possession of the claimed plasmids at the time of the application was filed. The picture on the last page of the copies of the original notebook is the same as the FIG. 2 presented in the application. Lane number 12 is the picture of the linearized plasmid p4C. These

copies of the original Oligo Data Sheet and original notebook provide clear evidence that the applicant, at the time of the application was filed, had possession of the claimed invention.

The specification clearly discusses the synthesis of SEQ ID NO: 37 or set forth its components. In paragraph 2 of page 8 under the DETAILED DESCRIPTION OF THE INVENTION, it is clearly described that the claimed plasmid combined from at least two DNA fragments comprising (a) to (e). On the paragraph under "Prepare a linear DNA fragment containing replication origin" of EXAMPLE 1 on page 13 of the application, the experimental conditions for preparing the DNA fragment containing replication origin were clearly described. On the paragraph under "Prepare a linear DNA fragment containing selection marker gene" of EXAMPLE 1 on page 13 to 14 of the application, the experimental conditions for preparing the DNA fragment containing selection marker gene were described. In paragraph 2 of page 14, linker sequences to link these DNA fragments were described. In paragraph 3 of page 14 under EXAMPLE 1 of the application, the detailed experiments about combining the DNA fragments containing the replication origin and selection marker gene were referred to the publication by the applicant (Li et al., Nucleic Acid Res. 25: 4156-4166 (1997)). The detailed experimental conditions were described in the reference. The first sentence of last paragraph of page 14 under EXAMPLE 2 states that "Sixty de novo synthesized plasmids were constructed as describe above". In paragraph 1 of page 15 under EXAMPLE 2, it is clearly described that "the plasmid p4C contains replication origin 4 and selection marker gene resistant to chloramphenicol". The DNA sequences of plasmid p4C are described in SEQ ID NO: 37 (last paragraph of page 14 under EXAMPLE 2). The last sentence of paragraph 1 of page 16 under EXAMPLE 2 describes that "PCR with oligo 1, oligo 4, and pACYC177 produced replication origin 4 DNA fragment based on p15A". The second paragraph of EXAMPLE 1 on page 13 contains a detailed description of making replication origin 4. The sequences of the oligos for synthesizing replication origins were described in the SEQUENCE LISTING. The last sentence of paragraph 1 of page 16 under EXAMPLE 2 describes "PCR with oligo 1, oligo 4, and pACYC177 produced replication origin 4 DNA fragment based on p15A". This described how replication origin 4 was synthesized.

Replication origin 4 is synthesized from plasmid pACYC177 to one skilled in the art. The first sentence of paragraph 2 of page 16 under EXAMPLE 2 describes that "Oligos 5 to 12 (sequence ID NOS: 5 to 12) were synthesized for selection marker genes of these ten plasmids". The sequences of the oligos for synthesizing selection marker genes were described in the SEQUENCE LISTING. The sixth sentence (lines 9 to 10) of paragraph 2 of page 16 under EXAMPLE 2 describes that "PCR with oligo 8, oligo 12, and pACYC184 produced chloramphenicol DNA fragment that will confer chloramphenicol resistance". This described how DNA fragment resistant to chloramphenicol was synthesized. Chloramphenicol resistant DNA fragment is synthesized from plasmid pACYC184 to one skilled in the art. As stated at the beginning of EXAMPLE 2, sixty plasmids including this p4C SEQ ID NO: 37 were constructed as described in EXAMPLE 1. It is clear that the plasmid is fully described in the specification, and therefore it has a patentable function. Therefore the link has been established between the claimed sequence SEQ ID NO: 37 and the replication origin of plasmid pACYC177 and chloramphenicol selection marker gene of plasmid pACYC184. Lane 13 of Fig. 2 in the application is the actual picture of the linearized plasmid p4C. The sequence of plasmid p4C is disclosed as SEQ ID NO: 37 in the SEQUENCE LISTING of the application. These are the direct evidences in the specification that the applicant, at the time of the application was filed, had possession of the claimed invention. Furthermore, the data of FIG. 2 demonstrating the copy number of the claimed plasmid has been presented. FIG. 2 shows the picture of the synthesized plasmids run on a 0.8% agarose gel. The paragraph 2 (lines 4 to 9) page 15 under EXAMPLE 2 clearly described FIG. 2. As described in the specification, all plasmids presented in FIG. 2 "were prepared under same conditions". As will be understood by one skilled in the art, the DNA quantities of the plasmids presented in the FIG. 2 will represent the copy number of these plasmids. The staining intensity represents the quantity of the DNA fragment. The higher the staining intensity the higher quantity the DNA fragment is. The relative staining intensities of the DNA fragments represent relative quantities and relative copy numbers of the linearized plasmids. The specification clearly describes plasmid p4C SEQ ID NO: 37 which was constructed from DNA fragments obtained by PCR containing replication origin from pACYC177 and chloramphenicol selection marker from pACYC184. One skilled in the

art will expect the copy number of plasmid p4C will be low copy number as pACYC177. However FIG. 2 clearly demonstrates that p4C has higher copy number than plasmid p2K as it would be expected for plasmids with replication origin obtained from low copy number plasmids such as pACYC177 or pACYC184. Surprisingly the copy number of p4C is higher than that of the plasmid p1A which contains replication origin prepared from plasmid pBR322. One skilled in the art would expect that plasmids with replication origin from plasmid pBR322 have higher copy number than the plasmids with replication origin from plasmid pACYC177. Furthermore, the copy number of plasmid p4C is comparable to the plasmid p3A, which contains replication origin prepared from plasmid pUC19. One skilled in the art will recognize that the copy number of a plasmid is one of the most important functions of a plasmid. The claimed plasmid p4C, which has unexpected high copy with replication origin from pACYC177 which is compatible with plasmids with replication origin from pBR322 or pUC19, will be useful in plasmid DNA production, protein co-expression and other biomedical applications. Therefore the data in FIG. 2 demonstrating the function of the claimed plasmid p4C has been clearly presented. One skilled in the art would definitely recognize that the applicant was in possession of the claimed invention, and as such the claimed invention presents an adequate written description.

The claim(s) contains subject matter which was described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The specification describes all components and some known functions of the claimed plasmid p4C SEQ ID NO: 37. The first sentence of paragraph 1 of page 15 under EXAMPLE 2 clearly describes that "p4C contains replication origin 4 and selection marker gene resistant to chloramphenicol". The last sentence of paragraph 1 of page 16 under EXAMPLE 2 describes that "PCR with oligo 1, oligo 4, and pACYC177 produced replication origin 4 DNA fragment based on p15A". One skilled in the art will recognize that a plasmid with a replication origin based on p15A will be compatible with plasmids with replication origins based on pBR322 or pUC19. One skilled in the art will also

recognize that a plasmid with a replication origin based on p15A can be used in protein co-expression with plasmids based on replication origins of pBR322 or pUC19. Lane 12 of FIG. 2 shows the data demonstrating relative high copy number of the claimed plasmid p4C. One skilled in the art will recognize that a plasmid with relative high copy number can be used in the plasmid DNA production. The sixth sentence (lines 9 to 10) of paragraph 2 of page 16 under EXAMPLE 2 describes that "PCR with oligo 8, oligo 12, and pACYC184 produced chloramphenicol DNA fragment that will confer chloramphenicol resistance". The produced chloramphenicol DNA fragment determines and predicts that a particular protein or polypeptide merely from the primary sequence or the nucleotide sequence encoding it as presented in SEQ ID NO: 37. This protein or polypeptide will be produced once the claimed plasmid is introduced in a host cell and this protein or polypeptide will enable the host cell to resist antibiotic chloramphenicol. The fact that plasmid p4C was made, at the time of the application was filed, proves the claimed plasmid contains a selection marker gene resistant to chloramphenicol. One skilled in the art will clearly recognize that the disclosed plasmid p4C will confer chloramphenicol resistance to its host cell. This is one of the functions of the claimed plasmid. In addition, the claimed plasmid also contains linker sequences. The composition and function of the linker sequences are described in the second paragraph on page 14 under EXAMPLE 1. The exact DNA sequences of the linker sequences are presented in SEQ ID NOS: 1, 4, 8, and 12. Therefore the specification describes all the components and some known functions of the claimed plasmid.

The specification contains information about the plasmid or its manner of usage or mode of operation is set forth. Examples of the use of the plasmids are also presented. The specification was written to disclose synthetic novel plasmids, the methods of making and use thereof. The DNA sequences of ten synthetic novel plasmids were disclosed in the SEQUENCE LISTING with SEQ ID NOS: 32 TO 41. All the components and some known functions of these plasmids are described. The making of all these plasmids is clearly described. The use of these plasmids is described as well. The method of using the synthetic plasmid is described on the last paragraph of page 10 under DETAILED DESCRIPTION OF THE INVENTION comprising steps (a) to (e). The following

paragraphs further describe the use of these plasmids. The description of the use of these plasmids can be applied to each or all of these disclosed plasmids including the claimed plasmid p4C SEQ ID NO: 37. Each of these disclosed plasmids contains two linker sequences. All ten plasmids contain the same two linker sequences as disclosed in SEQ ID NOS: 1 to 12. The two linker sequences each have two DNA strands. The DNA sequence of one strand is different from the complementary strand since the two linker sequences are not palindrome sequences. Since all these plasmids have the same linker sequences, all plasmids can be linearized and other DNA fragments can be cloned in the same way as in one linker sequences. Therefore the description of the use of these plasmids applies to all of these disclosed plasmids including the claimed plasmid p4C SEQ ID NO: 37.

The application disclosed a novel synthetic plasmid in general and 10 novel synthetic plasmids in detail. All components and some known functions of these 10 plasmids are fully disclosed including their sequences. With the disclosed application and incorporated references herein, one skilled in the art can easily make all these 10 plasmids and unlimited number of novel synthetic plasmids without empirical or undue experimentation. The applicant estimates that it will take one skilled in the art to order the synthetic oligos in one day, to perform the PCR and link the PCR DNA fragments in one day, and to obtain the plasmid DNA in one day. There shall be no mistake in oligo synthesis, PCR, or linking the PCR DNA fragments with disclosed application and references herein. One skilled in the art will recognize that all these molecular biology works of making these plasmids are easy, short and straight forward with disclosed specification. Three examples of using these plasmids are also fully described in the application. These examples will work for the plasmids presented in the examples and all 10 disclosed synthetic plasmids. One skilled in the arts will be able to use all 10 disclosed plasmids easily. The applicant believes the examples provided in the specification are sufficient for using all disclosed plasmids. Providing multiple examples for all 10 disclosed plasmids will be redundant and violates the requirement of conciseness in the first paragraph of 35 U.S.C. 112.

As matter of fact, each of the disclosed 10 plasmids is synthesized with oligonucleotides and PCR DNA fragments only. All required oligonucleotides are disclosed in the specifications. The PCR conditions and the templates for PCR DNA fragments of each plasmid are described in the specifications. Assembly of each plasmid is also described and referred in the specifications. The EXAMPLE 2 of the specifications describes the Construction of the ten disclosed plasmids. The picture of the gel containing the ten disclosed plasmids is shown on Fig. 2. Fig. 2 provides the evidence that the inventor had possession of the claimed plasmids at the time the application was filed. Fig. 2 also shows some functions of disclosed plasmids. One of the functions of the disclosed plasmids is that each plasmid confers a specific antibiotic resistance to its host cell. Each host cell was selected by an appropriate antibiotic during the plasmid construction. The fact that these plasmids were obtained from the selection process proves that these plasmids confer resistance to their respective antibiotics. Another function of the disclosed the plasmids is the autonomous replication of the plasmids in their host cells. The fact that these plasmids were obtained proves that these plasmids can autonomously replicate in their host cells. Fig. 2 together with the disclosed sequences shows that these disclosed plasmids are small in size. Extensive search of published data by the applicant indicates that these plasmids may be the smallest plasmids containing the same selection marker and replication origin. One skilled in the art can immediately recognize that smaller plasmids are advantageous in biomedical applications since they drain less cellular energy in their host cells. The conserved cellular energy can be directed to other desired DNA and protein synthesis. In addition, Fig. 2 also shows the relative copy number of the disclosed plasmids. One skilled in the art knows that both low and high copy numbers of the plasmids are useful for different biomedical applications. Therefore Fig. 2 proves that the applicant had the possession of all disclosed plasmids, it also show some functions of the disclosed plasmids. The demonstrated functions of the disclosed plasmids on Fig. 2 are convincing evidences to one skilled in the art that these plasmids are indisputably useful in different biomedical applications.

Fig. 1 in the application is a schematic diagram of construction of the synthetic plasmid p4T. This diagram can be changed to show p4C or any disclosed plasmid. Description of

this diagram can be changed accordingly. These changes will not reflect any material change of the application. No new matter will be introduced with these changes. As a matter of fact, many scientists do not draw a schematic diagram when making a plasmid. The applicant did not draw a schematic diagram on the original notebook when synthesizing the disclosed novel plasmids. The disclosed novel plasmids including plasmid p4C are synthesized with synthetic oligos and PCR DNA fragments only. All the synthetic oligos are described in the specifications. The conditions to synthesize all the PCR DNA fragments are clearly described in the application. The conditions to assemble the synthetic plasmids are also described and referred in the application. What important are the descriptions of the oligos, conditions of synthesizing the PCR DNA fragments and assembling the plasmids. These descriptions are for all disclosed plasmids. With these descriptions, all disclosed plasmids can be synthesized without the schematic diagram Fig. 1. Schematic diagrams of making plasmids are generally not shown in recent scientific publications. If the examiner feels a schematic diagram showing plasmid p4C is needed, the modified Fig. 1 showing a schematic diagram of construction of the plasmid p4C is included in this amendment for the examiner's consideration. The description of the Fig. 1 may be changed accordingly.

Fig. 2 in the application shows the actual picture of the claimed plasmids electrophoresized on a gel. It is the evidence that the applicant had the possession of the claimed plasmid at the time the application was filed. Fig. 2 also shows the functions of the claimed plasmids. Relative copy numbers of the claimed plasmids are shown as staining intensities in Fig. 2. Fig. 2 shows that the claimed plasmid p4C with SEQ ID NO: 37 is a relative high copy number plasmid. The fact that p4C was obtained indicates plasmid p4C is resistance to antibiotic chloramphenicol. The specifications clearly described that the replication origin of plasmid p4C is synthesized from pACYC177 plasmid. One skilled in the art will recognize that plasmid p4C will be compatible with plasmids with replication origins from pBR322 or pUC19. One skilled in the art will recognize that plasmid p4C will be useful in protein co-expression. In addition, one skilled in the art will also recognize that plasmid p4C will be useful in plasmid DNA

production because of its relative high copy number. Therefore Fig. 2 clearly shows the functions and applications of the claimed plasmid p4C.

The application was written to teach a novel synthetic plasmid, the methods of making and use thereof. Ten novel plasmids were given to show the novel synthetic plasmid. Examples were given to show the use of the novel plasmids. These examples can be applied to plasmid p4C as well. Fig. 1 shows schematic diagrams of construction of the de novo synthesized plasmid p4T. This figure may be changed to show any of the ten disclosed plasmids including p4C with no new matter introduced. At the time the application was filed, the disclosed plasmid p4C had been used in different biochemical applications. If the examiner feels applications of plasmid p4C should be included in the application, witnessed records of these applications can be supplied in the specifications with the examiner's instruction.

The specification describes four replications origins and four selection markers. The combination of these replication origins and selection markers can produce at least 16 plasmids. The actual number of combinations is much greater than 16 since each replication origin and selection marker have relative orientation. In addition, each plasmid also contains two linker sequences and each linker sequence has relative orientation. These facts make the number of combinations even greater. A plasmid is the smallest selectable autonomously replicating DNA genome. After extensive search of prior arts, it appears that the disclosed application is the first to teach a novel selectable autonomous replicating synthetic DNA genome, to make a novel synthetic DNA genome by combination of DNA fragments, and to use a novel synthetic DNA genome for biomedical applications. The disclosed technologies can be used to make larger selectable autonomous replicating genomes. The specification disclosed in the application may be the pioneer works in synthetic biology. Therefore the disclosed application may be one of the most important publications in the field of synthetic biology.

In conclusion, the application describes a de novo synthesized plasmid in general and ten synthesized novel plasmids in particular. All these ten plasmids are adequately and

clearly described in the specification. With disclosed invention, one skilled in the art will recognize the inventor had possession of the claimed invention, will be able to easily make and use the claimed invention.

The Claim 26 is written to direct the plasmid to disclosed SEQ ID NO: 37 which is the sequence of the disclosed synthetic plasmid p4C. The sequence, all components, and some known functions of plasmid p4C are disclosed in the specification. Therefore the claims are submitted that patentable subject matter is clearly presented. If the examiner agrees but does not feel that the present examples and figures are technically adequate, applicant will respectfully make the changes that the examiner instructs.